UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: February 28, 2001

MEMORANDUM

SUBJECT: **Oxadiazon:** Assessment of Mode of Action on Liver Carcinogenicity

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THRU: Pauline Wagner, Co-Chair
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and

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TO: William Burnham, Senior Scientist Advisor
Chairman, Cancer Assessment Review Committee (CARC)
Immediate Office
Health Effects Division (7509C)

cc: Anna Lowit, Executive Secretary, MTARC
Veronique LaCapra, Chemical Review Manager, SRRD
Branch Files

Oxadiazon

PC Code: 109001
DP Barcode: D266361

Action: The Mechanism of Toxicity Assessment Review Committee (MTARC) met on February 8, 2001 to evaluate the mechanistic and other relevant data to determine whether the available findings support peroxisome proliferation as a possible mechanism of action for liver tumors induction by Oxadiazon. The mechanistic data included one 14-day oral study in rats submitted by the Registrant (MRID No. 42310001) and a journal article (Richert *et al.*, 1996).

Conclusions: Based on the weight-of-the-evidence, there are sufficient data to classify Oxadiazon as a non-genotoxic hepatocarcinogen. The available data also provide suggestive evidence of peroxisome proliferation. There are, however, weaknesses in the database that preclude acceptance of peroxisome proliferation as the mode of action for Oxadiazon-induced liver tumors; these include:

(1) No cell proliferation data were reported for rats or mice; hence, mitogenesis could not be mechanistically linked to proliferation of peroxisomes.

(2) There was no convincing concordance between the dose response for peroxisomal enzymatic activity and tumor formation.

(3) The role of decreased catalase activity, which generally increases in the presence of a proxisome proliferator, was not explained by the investigators of the submitted study.

The Committee concluded, therefore, that peroxisome proliferation may be a possible mode of action for Oxadiazon-induced liver tumors in rats and mice. However, because of shortcomings in the database, the available information do not support this proposed non-genotoxic mode of action for Oxadiazon at this time.

Committee Members in Attendance

Members who were present and gave electronic concurrence to this report were: Karl Baetcke, Mike Ioannou, Anna Lowit, Alberto Protzel, and Pauline Wagner

Data evaluation prepared by: Nancy McCarroll, Toxicology Branch

Also in attendance were: Veronique LaCapra of SRRD and HaJung Sung of Rural Development Administration, Korea.

Proposed Mechanism of Action for Oxadiazon: Recommendations to the MTARC

I. Background: The relevance of peroxisome proliferation (PP) to hepatocarcinogenesis has been previously discussed (see HED Memorandum: Lactofen: Assessment of Mode of Action on Liver Carcinogenicity from Robert F. Fricke to Christine Olinger, dated February 15, 2001). Within this document, criteria are presented that must be satisfied before a non-genotoxic hepatocarcinogen can be classified as a PP. These criteria, which were established through the joint efforts of MTARC and CARC, are:

1. Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and an increased number of peroxisomes as measured by morphometric analysis.

2. Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

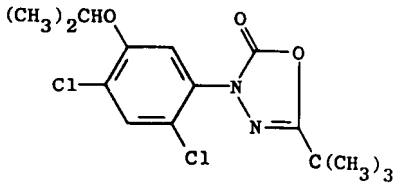
3. Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially acyl or palmitoyl CoA oxidase.

II. Physical and Chemical Properties of Oxadiazon

Oxadiazon, 2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-5-one, is a selective pre-emergent and early post emergence herbicide that is effective primarily for the control of annual grasses and broadleaf weeds in turf. The trade name for Oxadiazon in the U.S. is *Ronstar*. Oxadiazon has no food or feed uses. Most of the usage is allocated to golf courses. However, the Registrant is now supporting use of Oxadiazon on golf courses, apartment/condo lawns, athletic fields, parks, playgrounds, and cemeteries.

The mechanism of action is contact inhibition by affecting young shoots as they grow through the treated zone (pre-emergence) and complete coverage (post-emergence). Oxadiazon destroys cell membranes and inhibits photosynthesis, probably by generating oxidizing radicals in light and is a powerful inhibitor of plant, yeast and mouse protoporphyrinogen oxidase, an enzyme critical in the biosynthesis of chlorophyll and heme (Matringe *et al.*, 1989).

Oxadiazon has the following structure:



Empirical Formula: C_{15} H_{18} Cl_2 N_2 O_3

III. Classification of Carcinogenic Potential

According to the Cancer Assessment Review Committee (CARC) report, dated August 27, 1987 (HED Document No. 007798), the original peer review (November 21, 1984, HED Document No. 004097) placed Oxadiazon into Group B2 (probable human carcinogen) but there was a minority opinion that the agent should be placed in **Group C (possible human carcinogen).** Review of the weight-of-the-evidence on Oxadiazon by the Scientific Advisory Panel (dated November 20, 1987) reiterated this minority view. Consequently, the current Agency decision on the carcinogenic potential of Oxadiazon concurs with the Scientific Advisory Panel's (SAP) classification of Oxadiazon as a Group C carcinogen and the **Q_1* has been set at 1.4 x 10^{-1}(mg/kg/day)$^{-1}$ in human equivalents.** The rationale for the original classification as group B2 was based on the increased incidence of malignant or combined malignant and benign liver tumors: a) in multiple species (CD -1 mice and F344 rats of one or both sexes) and in multiple experiments (liver tumors in two mouse studies and in one rat study). **The decision to reclassify Oxadiazon as a Group C carcinogen was based on the rationale that liver tumors were produced in two of the three positive studies (one mouse study and one rat study) at doses that exceeded the maximum tolerated dose (MTD).**

Since the time of the classification of the oncogenic potential of Oxadiazon as a Group C carcinogen, a new chronic/oncogenicity study in rats (MRID No. 40993401) and a new carcinogenicity study in mice (MRID No. 40993301) have been submitted to the Agency. **The Hazard Identification Assessment Review Committee (HIARC) recommended that the Q_1* be revisited and that the Cancer Assessment Review Committee (CARC) reconvene to evaluate these more recent studies.** The CARC is awaiting the outcome of the current MTARC deliberations before evaluating these more recent chronic studies.

IV. Mutagenicity

Oxadiazon is neither mutagenic nor clastogenic but does cause neoplastic cell transformation *in vitro*. Acceptable bacterial assays with ≥97.49% Oxadiazon were negative for gene mutations in *Salmonella typhimurium* and *Escherichia coli* (MRID Nos. 00069893 and 41871701). Similarly, neither 95.5% Oxadiazon nor recrystallized Oxadiazon (100%) were mutagenic or clastogenic in cultured mammalian cells and did not cause unscheduled DNA synthesis (UDS) in primary rat hepatocytes. There is, however, evidence that both formulations induced neoplastic transformation in Syrian hamster kidney cells both in the presence and in the absence of S9 activation. The finding of positive cell transformation supports the evidence from mouse bioassays (MRID Nos. 00444322, 00115733 and 40993301) and the rat long-term studies (MRID Nos. 00149003/00157780 and 40993401) of liver tumor induction.

V. Evaluation of the Toxicology Database for Peroxisome Proliferation as a Possible Mechanism of Action for Liver Tumors Induced by Oxadiazon

Data submitted by the Registrant are from both guideline and one non-guideline (mechanistic) studies. In general, the database for Oxadiazon is complete and has been evaluated with respect to the potential of Oxadiazon to induce liver tumors via peroxisome proliferation. Results from submitted studies were selected to illustrate findings pertinent to peroxisome proliferation as the proposed mechanism of action of Oxadiazon and are summarized in Table 1. In addition, a published study conducted in rats, mice

and dogs (Richert *et al.*, 1996) has also been considered with pertinent results presented in Table 1. All data have been assessed relative to the peroxisome proliferation criteria presented earlier.

A. Criterion 1: Increased Liver Weights and Increased Peroxisome Proliferation

As shown in Table 1, increased absolute and relative liver weights were seen in both sexes of several rat strains (Sprague-Dawley, ♂ only examined, Fischer 344, CD, Wistar) in male and female mice (CD-1 and ICR-JCL) and Beagle dogs. The length of exposure ranged from 14 days to 2 years. Doses causing increased liver weights in rats ranged from ≥200 mg/kg/day (14 days), 51 mg/kg/day (6 months) to 6 mg/kg/day (24 months). In mice, the earliest time that liver weights were recorded in the Guideline studies was 52 weeks. At this interval, significant effects were seen at 113 mg/kg/day. By 104 weeks, liver weight increases were noted at doses ≥12 mg/kg/day. Similar results of significantly increased absolute and relative liver weights have been shown by Richert *et al.*(1996) in Sprague-Dawley rats (≥200 mg/kg/day, 14 days) and CD-1 mice (≥200 mg/kg/day, as early as 28 days post--treatment) but not in Beagle dogs after 28-days of exposure. Regardless of the rodent species, hepatomegaly was generally more pronounced in males than in females. Hence, the data show convincing evidence pointing to hypertrophy as the cause of hepatomegaly in rats and mice. There is also evidence of hyperplasia in CD-1 mice but it was only seen in one of two studies conducted with this mouse strain and was not reported in strain ICR-JCL.

In the 14-day oral mechanism study (MRID No. 42310001) with Sprague Dawley rats, electron micrographs showed an increase in peroxisomes at 500 mg/kg/day Oxadiazon. Livers from lower dose groups were not examined microscopically. However, electron microscopy of rat and mouse liver sections in the study of Richert *et al.* (1996), showed a qualitative and dose-dependent increase in peroxisomes at 20 mg/kg/day (minimal in 5 of 11 mice), 100 mg/kg/day (moderate in 7 of 7 mice) and 200 mg/kg/day (severe in 9 of 9 mice). Eight of 10 rats administered 500 mg/kg/day Oxadiazon also showed a "severe" increase in peroxisomes.

B. Criterion 2: Evidence of Cell Proliferation

As stated above, there is ample evidence of increased relative liver weights; however, no study has been submitted on the effects, if any, of Oxadiazon on replicative DNA synthesis. It is of note that Oxadiazon was tested in two *in vitro* assays (MRID Nos. 00115723 and -27) for UDS in primary rat hepatocytes. Both studies were negative for UDS, and cells undergoing DNA replication rather than DNA repair were reported to be evenly distributed among all groups.

C. Criterion 3: Evidence of Increased Enzymatic Activity

Increased levels of enzymes associated with liver toxicity (ALP, SGOT, SGPT and/or LDH) were recorded in subchronic dietary studies lasting 90-days in rats as well as chronic studies lasting 1-2 years in rats, mice or dogs and can be seen in many of the studies listed in Table 1. In the 14-day oral mechanism study (MRID No. 42310001) with Sprague Dawley rats, dose-related increases in palmitoyl CoA oxidation (PAO), palmitoyl carnitine transferase and acetyl carnitine

transferase (ACT) were reported at 20, 200 and 500 mg/kg/day; effects at ≥200 mg/kg/day were significant. Catalase activity was, however, significantly reduced at 200 and 500 mg/kg/day. This decrease in catalase activity, which is generally increased in the presence of peroxisome proliferation, was not explained by the investigators.

In agreement with the peroxisomal enzyme activity results from the submitted 14-day study, Richert *et al.* (1996) found a significant ($p<0.05$) and dose-related increase in rat (PAO) and ACT at 200 and 500 mg/kg/day. In mice, the same investigators noted that increases in PAO were achieved at 100 (174% of control) and 200 mg/kg ($p<0.05$). ACT was also significantly increased at these levels. No biochemical assays in dogs were performed. *In vitro* studies conducted as part of these investigations showed concentration dependent increases in both PAO and ACT at 2.5-10 x 10^{-5} M Oxadiazon in primary rat hepatocytes; no effect were seen in cultured human hepatocytes at comparable doses.

While increases in the activity of the two enzyme markers for peroxisome proliferation followed exposure to Oxadiazon, the concordance between the dose response for peroxisome enzymes and liver tumor induction is not strong. As shown in Table 2, a dose of 113 mg/kg/day induced significant ($p<0.01$) increases in adenomas (+23%) carcinomas (+41%) and adenomas/carcinomas combined (+63%) in male mouse livers while a comparable dose (100 mg/kg/day) caused only a moderate increase in the number of peroxisomes and a less than 2-fold increase in PAO. At 10.6 mg/kg/day in the oncogenicity study, adenomas (+17%) carcinomas (+16%) and adenomas/carcinomas combined (+33%) were also significantly increased. However, only a slight increase in the number of peroxisomes and no increased in PAO were seen.

Similar evidence of a weak response in peroxisome enzymes was note in male rats dosed with either 200 or 500 mg/kg/day Oxadiazon in the submitted mechanistic study or the published results of Richert *et al.*, (1996). Increases in PAO only ranged from 1.4- to 2.1-fold over control while liver tumor induction was achieved at 3.5 mg/kg/day. ACT activity, which is distributed not only in the peroxisomes but also in endoplasmic reticulum and mitochondria, only reached a 6-fold increase in male rats at 500 mg/kg/day. Although temporal associations were considered, the Committee acknowledged , based on its experience with peroxisome proliferators that increased peroxisome enzyme activity generally occurs (regardless of the time interval) at doses near the tumor doses. It concluded, therefore, that the findings with Oxadiazon indicate that tumorigenic doses were substantially lower than levels inducing peroxisome enzymes.

VI. Other Modes of Action

As stated previously, Oxadiazon is not mutagenic, and with the exception of peroxisome proliferation, no other mode of action has been hypothesized for Oxadiazon. However, a possible alternative mode of action could be oxidative damage to DNA through the production of hydrogen peroxide by increased fatty acid oxidation. This is because of the decreased catalase activity noted in the 14-day submitted study. Theoretically, the reduction in catalase activity could lower the degradation rate of H_2O_2 thus initiating increased levels of H_2O_2. Cattley *et al*. (1998) have hypothesized that since catalase levels "are never increased more than twofold an imbalance between generation and degradation of H_2O_2 within the peroxisome" would exist. The excess H_2O_2, that would result from a loss of catalase

(as demonstrated in the Oxadiazon study), could conceivably escape from the peroxisome, react with transition metals and form hydroxyl radicals. Cattley *et al.* note that if these radicals form in the proximity of macromolecules, oxidative damage may occur.

VII. Structural Activity Relationships (SAR)

Oxadiazon is not known to be structurally related to other known herbicides that are also peroxisome proliferators (Richert *et al.,* 1996); however, a wide variety of chemical classes have been shown to have the potential to induce peroxisome proliferation.

VIII. Conclusions

Based on the weight-of-the-evidence, there are sufficient data to classify Oxadiazon as a non-genotoxic hepatocarcinogen. The available data also provide suggestive evidence of peroxisome proliferation. There are, however, weaknesses in the database that preclude acceptance of peroxisome proliferation as the mode of action for Oxadiazon-induced liver tumors; these include:

(1) No cell proliferation data were reported for rats or mice; hence, mitogenesis could not be mechanistically linked to proliferation of peroxisomes.

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The Committee concluded, therefore, that peroxisome proliferation may be a possible mode of action for Oxadiazon-induced liver tumors in rats and mice. However, because of shortcomings in the data base, the available information do not support this proposed non-genotoxic mode of action for Oxadiazon at this time.

REFERENCE

Richert, L., Price, S., Chesne, C., Maita, K. Carmichael, N. (1996). Comparison of the induction of hepatic peroxisome proliferation by the herbicide oxadiazon *in vivo* in rats, mice, and dogs and *in vitro* in rat and human hepatocytes. Toxicol. Appl. Pharmacol 141: 35-43.

Cattley, R.C., DeLuca, J., Elcombe, C., Fenner-Crisp, P., Lake, B.G., Marsman, D.S., Pastoor, T.A., Popp, J.A., Robinson, D.E., Schwetz, B., Tugwood, J., Wahli, W. (1998). Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? Reg Toxicol and Pharm 27:47-60.

Matringe, M., Camadro, J.M., Labbe, P., Scalla, R. (1989). Protoporphyrinogen oxidase inhibition by three peroxidizing herbicides: oxadiazon, LS 82-556 and M&B 39279. FEBS Letters 245, number 1, 2:35-38.

Table 1 : Summary of Liver Effects with Oxadiazon

Study (MRID)	**Liver Weight**	**Liver Enzymes**	**Liver Histopathology (Nonneoplastic)**	**Liver histopathology (Tumors)**
14-Day Oral Peroxisome Proliferation - ♂ Rat (Sprague-Dawley) (42310001): 0, 20, 200, 500 mg/kg/day	Abs & rel wt ↑($p<0.05$) 200 & 500 mg/kg	($p<0.05$) and dose-related at 200 & 500 mg/kg ↓catalase,↑PAO, ↑palmitoyl carnitine transferase, ↑ACT, ↓G 6 PO_4ase at 500 mg/kg	At 500 mg/kg: ↑peroxisome proliferation; ↑lipids; ↑sinusoidal dilation & rough endoplasmic reticulum damage	NA
Oral Peroxisome Proliferation in ♂ Rat (Sprague-Dawley): 0, 20, 200, 500 mg/kg/day--**14 days (10/group)** ♂ **Mice** (CD-1): 0, 100, 200 mg/kg/day--**28 days (12/group)** ♂ **Dogs** (Beagles): 0, 500 mg/kg--**28 days (3/group)** (Richert *et al.*, 1996)	Abs & rel wt ↑($p<0.05$) ≥200 mg/kg/day--**rats** ≥100 mg/kg/day--**mice** NS ↑ 500 mg/kg/day--**dogs**	Dose-related ↑ PAO & ACT; ($p<0.05$) at 200 & 500 mg/kg/day--**rats** Dose-related ↑ PAO & ACT; ($p<0.05$) at 200 (PAO) & 200 & 500 mg/kg/day(ACT) --**mice**	At 500 mg/kg (only dose tested):↑peroxisome proliferation (severe in 8/10 -**rats** Dose-related ↑peroxi-some proliferation (minimal at 20 5/11; moderate at 100 7/7; severe at 200 mg/kg/day 9/9 -**mice**) No effects--**dogs**	NA

Abbreviations:
ALP = Alkaline phosphatase
SGOT = Serum glutamic-oxaloacetic transaminase
SGPT = Serum glutamic-pyruvic transaminase
LDH = Lactate dehydrogenase
PAO = Palmitoyl CoA oxidase
ACT = Acetyl carnitine transferase

Study (MRID)	Liver Weight	Liver Enzymes	Liver Histopathology (Nonneoplastic)	Liver Histopathology (Tumors)
90-Day Dietary ♂♀ Rat (CD) (00111804): 0, 25, 100, 1000 mg/kg/day	Abs & rel wt ↑($p<0.05$) 100 & 1000 mg/kg (♂♀)	**wks 4 & 13**:↑ALP (♂ 100& 1000 mg/kg; ♀1000 mg/kg), **wk 13:** ↑SGOT (♂ 100& 1000 mg/kg; ♀1000 mg/kg), **wks 4 & 13**: ↑SGPT(♂ 100& 1000 mg/kg; ♀1000 mg/kg)	At 1000 mg/kg (♂♀) & 100 mg/kg (♂): brown pig. Kupffer cells & bile canaliculi; marked **variability in cell size** and staining properties of hepatocytes & necrotic hepatocytes (Above findings generally seen in ≥90% ♂ & ≥60%♀)	NA
Chronic/Onco Rat (Fischer 344) (00149003/00157780): 0, 10, 100, 1000, 3000 ppm (≈ 0, 0.5, 4.8, 50.9, 163.1 mg/kg/day ♂; 0, 0.6, 5.9, 60.9, 192.7 mg/kg/day ♀)	Abs & rel wt ↑($p\leq0.05$-0.001) **6 mo**: 1000 (♂) & 3000 ppm (♂♀); **12 mo**: 1000 & 3000 ppm (♂♀); **24 mo**: 100, 1000 & 3000 ppm (♀♂)	**6 mo:**At ≥1000 ppm (♂) ↑ALP, SGPT-- (at 3000 ppm ♂)↑SGOT (at 3000 ppm ♀)↑SGOT, SGPT, LDH, ALP; **12 mo:** (at ≥1000 ppm ♂♀) ↑ALP, SGPT ; **24 mo:** (at 3000 ppm ♂) ↑ALP, LDH	Progressive alterations from **hypertrophy** through fatty changes to necrosis (≥1000 ppm ♂ & 3000 ppm ♂)	($p<0.05$) pairwise and trend ↑adenomas & carcinomas combined (≥1000 ppm ♂). No decrease in latency. Benign & malignant tumors after prolong-ed exposure to hepatotoxic doses

Study (MRID)	**Liver Weight**	**Liver Enzymes**	**Liver Histopathology (Nonneoplastic)**	**Liver histopathology (Tumors)**
Chronic/Onco Rat (Wistar) (40993401): 0, 0, 3, 10, 100, 1000 ppm (≈ 0, 0.1, 0.4, 3.5, 39 mg/kg/day ♂; 0, 0.1, 0.4, 4.2, 44 mg/kg/day ♀)	Abs & rel wt ↑(p≤0.05-0.001) **26 wk**: 1000 ppm (♂♀); **52 wk**: 1000 ppm (♂); **78 wk**: 1000 ppm (♀♂); **104 wk**: 1000 ppm (♂)	**wks 26**:↑LDH (♂ 1000 mg/kg **& wk 52**);↑ALP (♂ 1000 mg/kg); ↑SGOT/SGPT (♂ 1000 mg/kg). **Wks 52, 78 & 104:** No effects.	At 1000 ppm (p≤0.01-0.001): ↑ **centrilobular hepato. swell**.(♂♀); ↑ brown pigmentation in liver) (♂♀); ↑ foci of cell alteration (♂); ↑ bile duct proliferation) (♂). At 100 ppm (p<0.05) , ↑ **centrilobular hepato. swell**.(♂)	(♂) S trend (p<0.01) & S pairwise 100 & 1000 ppm (p<0.05-0.01) liver adenomas and/or carcinomas combined. At 1000 ppm, S pairwise (p<0.05) carcinomas .
Onco Mice (CD-1) **(00044322)**: 0, 300, 1000, 2000 ppm (≈0, 48, 153, 319 mg/kg/day ♂; 0, 62, 201, 417 mg/kg/day ♀)	Abs & rel wt ↑(p≤0.05-0.01) at **104 wk**: ≥300 ppm (♂♀)	**wk 104**: (p≤0.05-0.01)↑ALP (≥300 ppm ♀, ≥1000 ppm ♂); ↑SGOT (≥1000 ppm ♂♀);↑SGPT (≥300 ppm ♀, ≥1000 ppm ♂)	↑centrilobular **hypertrophy** (≥300 ppm ♂♀); ↑diffuse hepatocellular **hyperplasia & hypertrophy** (≥300 ppm ♂♀);↑ nodular **hyperplasia & hypertrophy** (≥300 ppm ♂♀);↑ focal necrosis (2000 ppm ♂♀)--no stats	p<0.01 ↑ hepatocarcinomas (≥1000 ppm ♂♀). No decrease in latency.

Study (MRID)	Liver Weight	Liver Enzymes	Liver Histopathology (Nonneoplastic)	Liver histopathology (Tumors)
Onco Mice (CD-1) **(00115733)**: 0, 100, 300, 1000, 2000 ppm (≈0, 12, 37, 122, 254 mg/kg/day ♂; 0, 14, 44, 143, 296 mg/kg/day ♀)	Abs & rel wt ↑(p≤0.05-0.001) at **105 wk**: ≥100 ppm (♂), ≥1000 ppm (♀)	Not Done	No detail; **terminal sac**: lesions listed as masses (all dose groups, both sexes), pale areas/foci (all dose groups, both sexes), raised areas (all dose groups, females only) **unscheduled deaths:** pigmented Kupffer cells, hepatic single cell necrosis (high-dose males)	p<0.05-0.01 ↑ liver adenomas (≥100 ppm ♂♀); p<0.05↑ carcinomas (≥100 ppm ♂ 1000 ppm ♀); p<0.05-0.01 ↑ combined liver adenomas & carcinomas (≥100 ppm ♂♀). p<0.05 trend for carcinomas (♂),adenomas (♀) & combined (♂♀)
Onco Mice (ICR-JCL) **(40993301)**: 0, 3, 10, 100, 1000 ppm (≈0, 0.3, 1, 11, 113 mg/kg/day ♂; 0, 0.3, 1, 9, 99 mg/kg/day ♀)	Abs & rel wt ↑(p≤0.05-0.001) at **52 & 98 wk**: 1000 ppm (♂), **98 wk**: 1000 ppm (♀)	**wks 52**:↑ALP (♂ 1000 mg/kg **& wk 98** ♂♀ 1000 ppm); ↑SGOT (♂ ≥100 ppm).↑SGPT (♂ ≥100 ppm **wk 52 & at wk 98**; ♀ 1000 mg/kg **at wk 52**)	<u>At 1000 ppm</u> (p<0.001): ↑ **centrilobular hepato. swell**.(♀); ↑**diffuse hepatocellular swell**.(♂♀); ↑ brown pigmentation (♂♀) ↑ diffuse hepatocellular necrosis (♂ only) at 1000 ppm (p<0.05) but (p<0.001) at 100 ppm. Also in ♂ <u>at 100 ppm</u>: (p<0.001) ↑**diffuse hepatocellular swell** and ↑brown pigment-ation (♂ only)	p<0.01 ↑ liver adenomas (≥100 ppm ♂, 1000 ppm ♂); p<0.01 ↑ liver carcinomas (≥100 ppm ♂, 1000 ppm ♂) p<0.01 ↑ liver adenomas/carcinomas combined (≥100 ppm ♂, 1000 ppm ♂) . ♂♀: p<0.05-0.01 trend for liver adenomas, carcinomas, and adenomas/carcinomas combined.

Study (MRID)	Liver Weight	Liver Enzymes	Liver Histopathology (Nonneoplastic)	Liver histopathology (Tumors)
1-Year Chronic Dog (Beagles) (41326401) 0, 5, 20, 60, 200 mg/kg/day	Abs & rel wt ↑($p \leq 0.05$-0.01) at **necropsy**: ♂: rel ≥60 mg/kg/day--abs at ≥60 mg/kg/day also ↑ but NS; ♀: abs ≥20 mg/kg/day--rel also ↑ but S only at 60 mg/kg/day	**wks 24 & 50**:↑SGOT (♂ 60 mg/kg ($p<0.05$)	↑ periacinar apoptosis centriacinar hepatocytic vacuolation	None
2-Generation Reproduction Rat (CD) (41239801) 0, 20, 60, 200 ppm (≈0, 1.5, 4.65, 15.5 mg/kg/day ♂; 0, 1.8, 5.6, 18.2 mg/kg/day ♀)	P adults: No effect F1 adults: Sli ↑ rel wt 200 ppm ♀ F1 and F2 offspring: Not measured	Not measured	P adults: No effects F1 adults: periacinar hepatocellular **hypertrophy** 200 ppm ♂ F1 or F2 offspring: No effects	None

Abbreviations:
ALP = Alkaline phosphatase
SGOT = Serum glutamic-oxaloacetic transaminase
SGPT = Serum glutamic-pyruvic transaminase
LDH = Lactate dehydrogenase
PAO = Palmitoyl CoA oxidase
ACT = Acetyl carnitine transferase

Table 2. Summary of Peroxisomal Effects and Liver Tumor Induction in Male Mice Administered Oxadiazon

Dose (mg/kg/day)	No. of Peroxisomes[a]	Peroxisomal Enzyme Activities[b]		Neoplasms[c]		
		Palmitoyl CoA oxidase (% ↑ over control)	Acetyl carnitine transferase (% ↑ over control)	Adenomas	Carcinomas	Adenomas/ Carcinomas Combined
0	0	--	--	2/69 3%	3/69 4%	5/69 7%
0.3	--	--	--	7/71 10%	1/71 1%	8/71 11%
1.1	--	--	--	2/71 3%	4/71 6%	6/71 8%
10.6	--	--	--	12/69** 17%	11/69* 16%	23/69** 33%
20	Slight (6/11)	106	113	--	--	--
100	Moderate (7/7)	174	389*	--	--	--
113	--	--	--	16/71** 23%	29/71** 41%	45/71** 63%
200	Severe (9/9)	259*	459.5*			

* Significantly different than control ($p<0.05$)
** Significantly different than control ($p<0.01$)

[a] Data were extracted from Richert *et al.* (1996).

[b] Data were extracted from Richert *et al.* (1996).

[c] Data were from Mouse 23- month chronic toxicity and oncogenicity study (MRID No. 40993301).